A SIMPLE HPLC-UV METHOD FOR THE ANALYSIS OF CIPROFLOXACIN IN TISSUE

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INTRODUCTION

The analysis of drugs in tissue is employed in various fields. Quantitative analysis of drugs in tissue may be applied to better relate concentrations with pharmacokinetic and pharmacodynamic responses and to understand therapeutic and toxic properties.[1]

There are no current validation guidelines which instruct and help the analyst with the homogenisation, extraction and analysis of drugs or molecules in solid samples.

Calculating actual recovery and stability of the analyte of interest in tissue can be described as being difficult.[2]

AIM

To validate a simple, robust, rapid and efficient High Performance Liquid Chromatographic method of analysis to quantify ciprofloxacin– a popular and widely used antibiotic in tissue.

METHOD

Pork tissue was spiked with six different concentrations of ciprofloxacin (0.05, 0.5, 1, 1.5, 2 and 2.5µg/ml) and the internal standard. Tissue was then homogenised using an electric tissue homogeniser (MSE® UK).

Protein precipitation using ice-cold acetonitrile was performed following the addition of two drops of buffer (pH 2.7) to the tissue homogenates.

Homogenates were mixed for one minute and centrifuged at 3,500g for 5 minutes. The organic layer was then evaporated of, the sample reconstituted,

re-centrifuged and the supernatant analysed using HPLC coupled with a UV detector.

Analysis was carried out using a Varian Pro Star HPLC unit. The UV-visible detector was set at 277nm. Separation took place at 25°C on an ACE® C_{18} column (250x 4.6mm; 5µm).

The mobile phase was made up of a 0.02M phosphate buffer (pH 2.7) and acetonitrile (77:23 v/v). The flow rate was of 1.5ml/min.

The method was validated for linearity, selectivity/ specificity, accuracy, precision, limit of detection, limit of quantification and stability.

RESULTS

The method was found to have acceptable linearity, with a regression coefficient of 0.9927. The method was selective to ciprofloxacin (Figure 1) and had a good limit of detection and quantification (0.05µg/ml).

Results for accuracy and recovery were acceptable. All percentage recoveries calculated were above 94%.

Relative standard deviation values for intra-day precision ranged from 2.25-12.68% whilst those for inter-day precision ranged from 3.03-11.49%, indicating that the method had an acceptable degree of precision.

Ciprofloxacin was found to remain stable in tissue when samples were analysed following storage at –20°C after 3 weeks and 6 weeks.

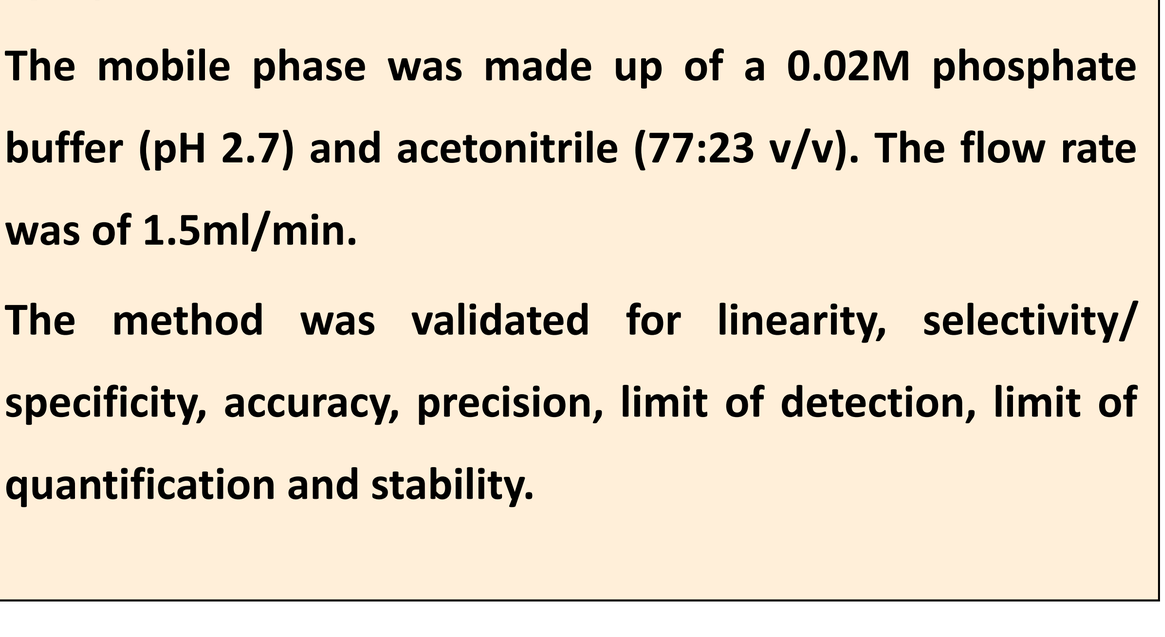

Figure 1: Chromatogram of Ciprofloxacin 2.5µg/ml (retention time: 3.15 minutes) with Sulfadimidine (retention time: 5.01 minutes)

CONCLUSION

The developed method provides efficient analysis of ciprofloxacin in tissue with a total run time of less than 6 minutes.

Application of this method can help quantify this antibiotic in tissue of patients suffering from diverse conditions.

References:

1. Timmerman P, Kall MA, Lasskso S, Freisleben A, Hucker R. Best practices in a tiered approach to metabolite quantification: views and recommendations of the European Bioanalysis Forum. Bioanalysis 2010;2(7): 1185-94
2. James CA, Breda M, Bartatté S, Casati M, Grassi S, Pellegatta B et al. Analysis of drugs and metabolites in tissues and other solid matrices. Chromatographia 2004; 59(2): 149-56